

Electronically Filed On: May 15, 2007

PATENT
Attorney Docket No. 34170-701.501

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application:

Inventor: Peter S. Lu et al.

Application No.: 10/630,590

Filed: July 29, 2003

Title: **METHODS OF DIAGNOSING
CERVICAL CANCER**

Confirmation No.: 4993

Examiner: Lucas, Zachariah

Group Art Unit: 1648

Customer No. 21971

DECLARATION UNDER 37 C.F.R. §1.131

We, Peter S. Lu, Johannes Schweizer, Chamorro Somoza Diaz-Sarmiento, and Michael P. Belmares, declare as follows:

1. We are the inventors of claims 1, 3-8, 10-22 and 24-28 of the patent application identified above and the inventors of the subject matters described and claimed therein.

2. We conceived and reduced to practice in this country the invention claimed in claims 1, 3-8, 10-22 and 24-28 in the above-referenced application prior to September 6, 2001.

3. Prior to September 6, 2001 we conceived that the E6 protein of oncogenic human Papillomavirus (HPV) has a C-terminal domain with a consensus sequence of -X-(S/T)-X-(V/I/L) that should be recognized and specifically bound to by a PDZ domain, such as domain 2 of MAGI-1, while non-oncogenic or low risk HPV E6 sequences should not. We developed assays to assess the binding interactions between the C-terminal domain of E6 protein of various oncogenic and non-oncogenic strains of HPV, such as the "G Assays" described in above-referenced application

serial No. 10/630,590 at pages 43-46, and in provisional application No. 60/309,841 filed on August, 3, 2001 at pages 32-36.

4. Prior to September 6, 2001 we designed and purchased from commercial suppliers peptides (see **Exhibit A** for evidence of purchase with dates obscured) containing the consensus C-terminal sequences derived from various oncogenic strains of HPV, and C-terminal sequences from non-oncogenic strains of HPV. **Table 1** below lists the sequences of such peptides with the C-terminal consensus sequences of oncogenic strains of HPV highlighted in bold. As listed in **Table 1**, besides peptides which have the native sequences of the C-termini of the HPV strains, we also designed peptides that are derived from the native sequences of the C-termini by substituting amino acid residues (especially cysteine residues) outside the consensus 4 amino acid C-terminal sequences with other amino acid residues to avoid complications resulting from aberrant folding of the native peptides due to cross-linking of the cysteine residues that causes aggregation of the peptides. See designed peptide sequences labeled with "(modified)" or "(cysteine-free)" in **Table 1**.

Table 1. Sequences of C-terminal peptides derived from E6 protein of various HPV strains.

AVC Name	Sequence	oncogenic
HPV E6 16	WTGRCMSCCRSSRTRRETQL	Y
HPV E6 16 (Modified)	TGRGMSGGRSSRTRRETQL	Y
HPV E6 18	HSCCNRARQERLQRRRETQV	Y
HPV E6 18 (Modified)	SGGNRARQERLQRRRETQV	Y
HPV-E6 31	GRWTGRCIACWRRPRTETQV	Y
HPV E6 33	CAACWRSARRRRRLQRRRETAL	Y
HPV E6 33 (modified)	AAGGRSARGGRLQGRRETAL	Y
HPV E6 35	GRWTGRCMSCWKPTRRETEV	Y
HPV E6 35 (cysteine-free)	GRWTGRAMSAWKPTRRETEV	Y
HPV E6 36 (cysteine-free)	RVRNAWKGIARQAKHFYNDW	N
HPV-E6 51	CANCWQRTQRRLQRRNETQV	Y
HPV E6 52	MGRWTGRCSECWRPRPVTQV	Y
HPV E6 52 (modified)	SEGGRPTRGPRLQGRRVTV	Y
HPV E6 57	HCMNCAPRCMENAPALRTSH	N
HPV E6 57 (cysteine-free)	HAMNAAPRAMENAPALRTSH	N
HPV E6 58	GRWTGRCACWCWRPRRRQTQV	Y
HPV E6 58 (modified)	AVGGRPARGGRLQGRRRQTQV	Y
HPV E6 63	VHKVRNKFKAQCSLCRLYII	N
HPV-E6 66	TGSLQCWRHTSRQATESTV	Y
HPV E6 66 (cysteine-free)	TGSALQAWRHTSRQATESTV	Y
HPV-E6 70	RHCWTSNREDRRRIIRRETQV	Y
HPV-E6 77	GHWRGSLHCWSRCMGQSRQ	N
HPV E6 77 (modified)	GGGRGSGLAGGSRGGGQSRQ	N
HPV-E6 80	QFHKVRNWKGLCRHCGSIE	N

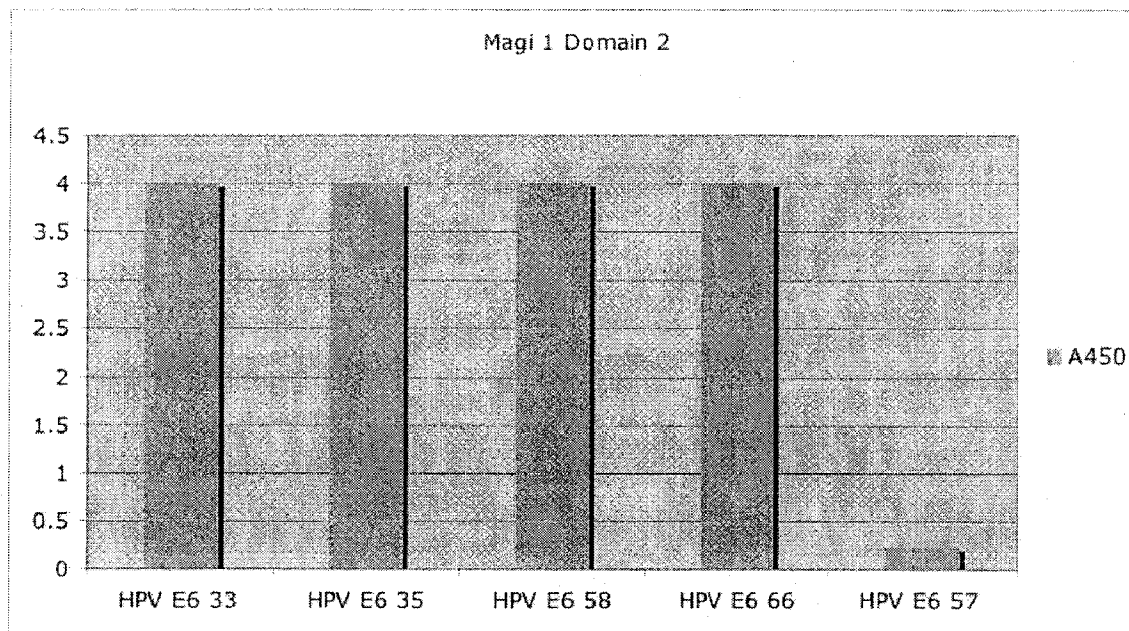
5. Prior to September 6, 2001 we used the G Assays to assess the interactions of peptides (**Table 2**) derived from the C-terminal 19-20 amino acids of E6 protein from oncogenic HPV types 33, 35, 58, 66 and non-oncogenic type 57. Peptide concentrations used in the G-assay were 10 uM, 1 uM, 10 uM, 3 uM, and 10 uM, respectively. **Figure 1** summarizes the results of these experiments with the C-terminal consensus sequences of oncogenic strains of HPV highlighted. The absorbance value at 450 nm indicates the amount of HPV peptides bound to MAGI-1 domain 2. **Exhibit B** is a copy of pages from lab notebooks recording such experiments on which the dates have obscured.

6. As shown in **Figure 1**, prior to September 6, 2001 we demonstrated that all four of peptides derived from the E6 protein of oncogenic HPV strains 33, 35, 58 and 66 bound MAGI-1 PDZ domain 2 strongly at 1-10 uM peptide concentrations. In contrast, the E6 sequence from non-oncogenic HPV type 57 did not bind to MAGI-1 domain 2. In addition, peptides derived from the E6 protein of oncogenic HPV strains 16 and 18 that share the same consensus C-terminal sequence as strains 33, 35, 58 and 66 were later demonstrated to bind to MAGI-1 domain 2. Thus, since the claimed invention is a method or system for detecting the presence of an oncogenic HPV in a sample by using a PDZ domain polypeptide of less than 1000 amino acids in length and comprising the amino acid sequence of MAGI-1 PDZ domain 2, the claimed invention was conceived and reduced to practice prior to September 6, 2001.

Table 2. Sequences of C-terminal peptides derived from E6 protein of various HPV strains.

HPV Type	E6 C-terminal sequence	Derived from Oncogenic HPV E6
HPV 33 (modified)	AAGGRSARGGRLQGRRETAL	Y
HPV 35 (cysteine-free)	GRWTGRAMSAWKPTRRETEV	Y
HPV 58 (cysteine-free)	AVGGRPARGGRLQGRRQTQV	Y
HPV 66 (cysteine-free)	TGSALQAWRHTSRQATESTV	Y
HPV 57 (cysteine-free)	HAMNAAPRAMENAPALRTSH	N

FIGURE 1: Binding strength of HPV E6 peptides with PDZ domain 2 of MAGI-1



7. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 05/10/2007

By: [Signature]

Peter S. Lu, M.D.
Country of Citizenship: U.S.A.

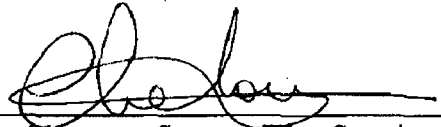
Date: 05/07/2007

By: [Signature]

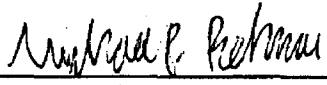
Johannes Schweizer
Country of Citizenship: Germany

U.S. Appl. Serial No. 10/630,590

Date: 5/8/07

By: 
Chamorro Somoza Diaz-Sarmiento
Country of Citizenship: Spain

Date: 5/07/07

By: 
Michael P. Belmares
Country of Citizenship: U.S.A.

Custom Peptide Synthesis Certificate of Analysis

Sequence Name: AA69.1 Scale: Research

Sequence:

Length: 19

N-Term	C-Term
N-TGR / GMS / GGR / SSR / TRR / ETQ /	L -- 'C'
C-Term	

Molecular Weight: 2094Quantity: 20 mg 0.0019 mmoleForm: Lyophilized powder

Analysis:

* HPLC

* Amino acid

* Mass spectroscopy

Storage and Stability: Stable for one year at -20 °C.Lot No. 10017449

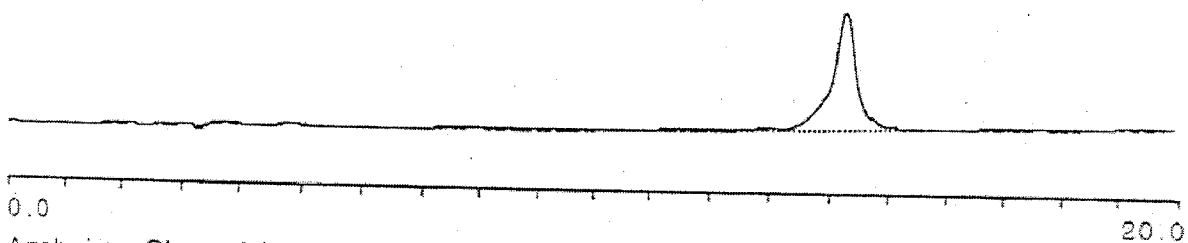
PLEASE SEE ATTACHED FOR QUALITY CONTROL DATA

Genemed Synthesis, Inc.

213 East Grand Avenue, South San Francisco, CA 94080 U.S.A.

Tel: 650-952-8193 Fax: 650-952-9540 www.genemedsyn.com

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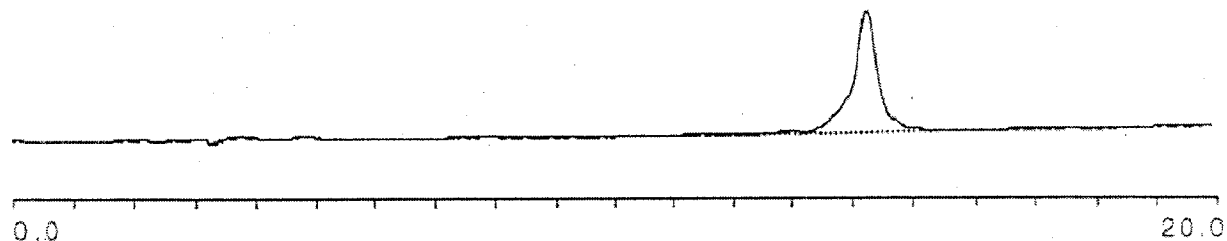
Analysis: Channel A

Peak No.	Time	Type	Height(μV)	Area(μV-sec)	Area%
1	14.255	N4	196364	6235630	100.000
Total Area				6235630	100.000

Data: 0-100 pepanal 006

Sample: 17449 25 μ l injected
Column: Vydac C18 1ml/min
Buffers: A=0.1%TFA; B=0.1%TFA in CH₃CN
Gradient: 0-100%B, 20'
Monitor: 220nm, 1.0 AUFS

Processing File: profile#1
Method: 0-100 pepanal
Inject Vol:
Sampling Int: 0.1 Seconds
Data:



Analysis: Channel A

Peak No.	Time	Type	Height(μ V)	Area(μ V-sec)	Area%
1	14.255	N4	196364	<u>6235630</u>	<u>100.000</u>
Total Area				6235630	100.000

Custom Peptide Synthesis
Certificate of AnalysisSequence Name: AA70.1 Scale: Research

Sequence:

N End:	C End:
N ¹ -SGG / NRA / RQE / RLQ / RRR / ETQ / V -- 'C	

Length: 19Molecular Weight: 2298Quantity: 20 mg 2.91X10⁻³ mmoleForm: Lyophilized powder.

Analysis:

* HPLC

* Amino acid

* Mass spectroscopy

Storage and Stability: Stable for one year at -20 °C.Lot No. 10017450

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2800 3000 3200 3400
s (m/z)

Laser: 2230

Mirror Ratio: 1.070

Scans Averaged: 61

PSD Mirror Ratio:

Pressure: 6.61e-07

Timed Ion Selector: 16.1 OFF

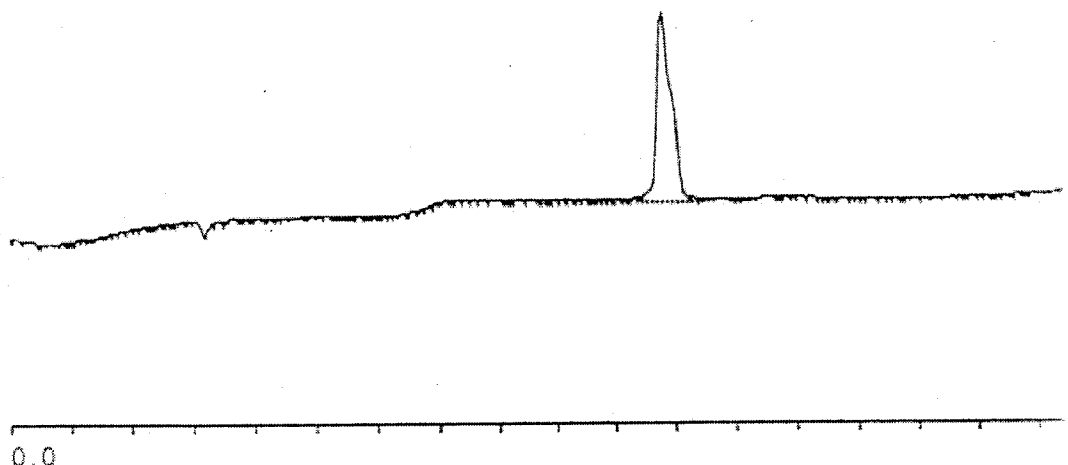
Low Mass Gate: OFF

Negative:

Date:
Data: 0-100 pepanar- 016

Sample: 17450 25 μ l injected
Column: Vydac C18 1ml/min
Buffers: A=0.1%TFA; B=0.1%TFA in CH₃CN
Gradient: 0-100%B, 20'
Monitor: 220nm, 1.0 AUFS

Processing File: profile#1
Method: 0-100 pepanal
Inject Vol:
Sampling Int: 0.1 Seconds
Data:



0.0

Analysis: Channel A

Peak No.	Time	Type	Height(μ V)	Area(μ V-sec)	Area%
1	10.780	N	96091	1355988	100
Total Area				1355988	100

Custom Peptide Synthesis

Certificate of Analysis

Sequence Name: AA72.1 Scale: Research

Sequence:

N' End:	C' End:
Biotin	
N--AAG / GRS / ARG / GRL / QGR / RET /	
AL--C	

Length: 20

Molecular Weight: 2267 2265.41 RE

Quantity: 20 mg 100 10 mmole

Form: Lyophilized powder.

Analysis:

- * HPLC
- * Amino acid
- * Mass spectroscopy

Storage and Stability: Stable for one year at -20 °C.

Lot No. 10017517

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Processing File: profile#1
Method: 0-100 pepanal
Inject Vol:
Sampling Int: 0.1 Seconds
Data:

Sample: 17517 25ul injected
Column: Vydac C18 1ml/min
Buffers: A=0.1%TFA; B=0.1%TFA in CH3CN
Gradient: 0-100%B, 20'
Monitor: 220nm, 1.0 AUFS
2

Data: 0-100
-014



Certificate of Analysis

Peptide Name: **AA80.1**

Run Number: **17702**

Sequence: **Biotin-Gly-Arg-Trp-Thr-Gly-Arg-Ala-Met-Ser-Ala-Trp-Lys-Pro-Thr-Arg-Arg-Glu-Thr-Glu-Val-OH**

Theoretical Mass(M+H⁺): ~~2603.0~~ **2600.51**

Mass Found(M+H⁺): **2602.3**

Solubility: **Dissolve 1mg of peptide in 1ml Water**

Appearance: **White Powder**

HPLC Purity: **> *N/A %**

Amount Delivered: **100 mg** *Customer requested unpurified peptide

Storage : **Keep Refrigerated**

Remarks: Not for Human Use. Research Purposes Only.

Release By: Jaswinder Kaur Date: _____

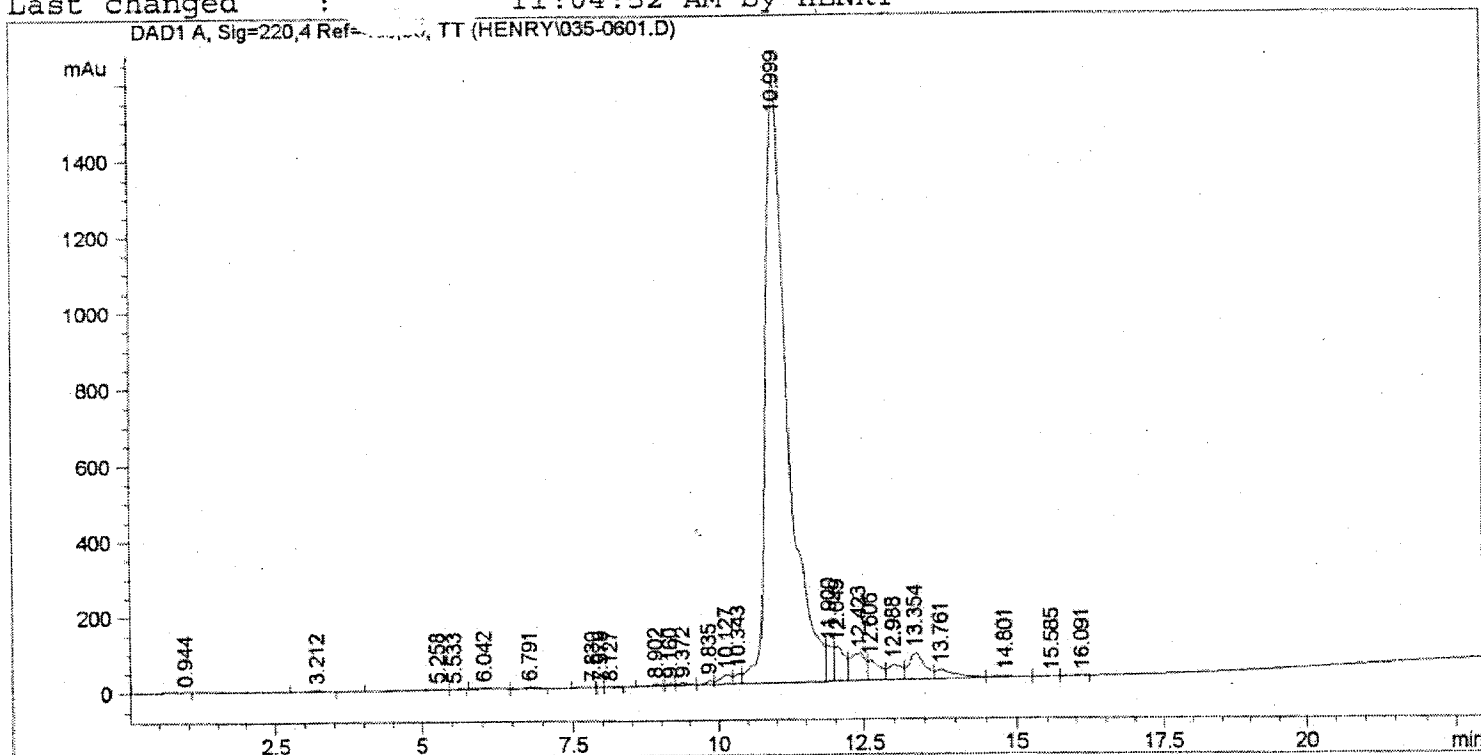
Quality Control

Sample Name: AA80.1

```

Injection Date      :                               Seq. Line :      6
Sample Name        : AA00.1                        Vial       :    35
Acq. Operator      : HENRY                          Inj        :     1
                                                    Inj Volume : 5 µl
Different Inj Volume from Sequence !      Actual Inj Volume : 2 µl
Sequence File      : C:\HPCHEM\1\SEQUENCE\DEF_LC.S
Method             : C:\HPCHEM\1\METHODS\0-100-20.M
Last changed       :      11:04:52 AM by HENRY

```



Area Percent Report

```
Sorted By      :      Signal
Multiplier    :      1.0000
Dilution      :      1.0000
```

Signal 1: DAD1 A, Sig=220,4 Ref=450,80, TT
Results obtained with standard integrator!

Peak #	RetTime [min]	Type	Width [min]	Area [mAu*s]	Height [mAu]	Area %
1	0.944	BV	1.0963	27.31302	2.94576e-1	0.0558
2	3.212	BV	0.2199	65.38581	4.18458	0.1336
3	5.258	PV	0.3324	38.73496	1.52027	0.0792
4	5.533	VB	0.1817	8.00819	7.34552e-1	0.0164
5	6.042	BV	0.1638	48.28724	4.21066	0.0987
6	6.791	PV	0.1581	51.54432	4.71076	0.1053
7	7.830	PV	0.2492	13.29615	6.67421e-1	0.0272
8	7.979	VV	0.0858	3.90877	6.33285e-1	7.988e-3
9	8.127	VB	0.1281	7.46610	7.92417e-1	0.0153
10	8.902	PV	0.1558	68.03886	5.92069	0.1390
11	9.160	VV	0.1115	43.50458	5.49541	0.0889

Custom Peptide Synthesis

Certificate of Analysis

7 ms 1007 MS 10:31 AM Sample: 45
Collected

Sequence Name: Scale: Research

N' End: Blothin	
N'--AVG / GRP / ARG / GRL / QGR / RQT /	
QV -- 'C'	
C' End:	

Sequence:

Length: 20

~~347~~ 2345-49

Molecular Weight: 2121

Quantity: 20 mg 25410 mmole

Form: Lyophilized powder.

Analysis:

- * HPLC
- * Amino acid
- * Mass spectroscopy

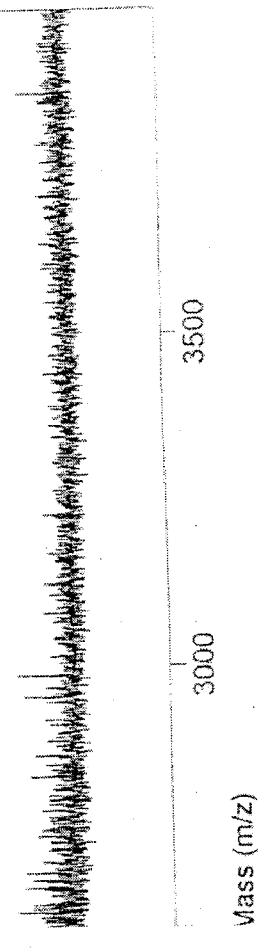
Storage and Stability: Stable for one year at -20 °C.

Lot No. 10017523

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Laser: 2190 Mirror Ratio: 1.070
Scans Averaged: 12 PSD Mirror Ratio:
Pressure: 8.00e-07 Timed Ion Selector: 16.1 OFF
Low Mass Gate: OFF Negative Ions: OFF

Date:

Data: C-100 pepanal- 007

Sample: 17523 25 μ l injected
Column: Vydac C18 1ml/min
Buffers: A=0.1%TFA; B=0.1%TFA in CH₃CN
Gradient: 0-100%B, 20'
Monitor: 220nm, 1.0 AUFS

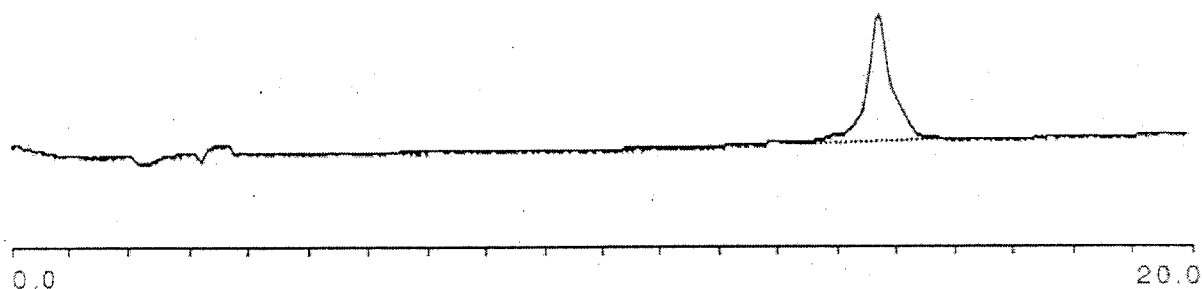
Processing File: profile#1

Method: 0-100 pepanal

Inject Vol:

Sampling Int: 0.1 Seconds

Data:



Analysis: Channel A

Peak No.	Time	Type	Height(μ V)	Area(μ V-sec)	Area%
1	14.721	N11	112398	<u>3014595</u>	<u>100.000</u>
Total Area				3014595	100.000



Certificate of Analysis

Peptide Name: **AA66.1**
Run Number: **17700**
Sequence: **BIOTIN-Thr-Gly-Ser-Ala-Leu-Gln-Ala-Trp-Arg-His-Thr-Ser-Arg-Gln-Ala-Thr-Glu-Ser-Thr-Val-OH**

Theoretical Mass(M+H⁺): **2414.7**

Mass Found(M+H⁺): **2414.3**

Solubility: **Dissolve 1mg of peptide in 1ml Water**

Appearance: **White Powder**

HPLC Purity: **> *N/A %**

Amount Delivered: **100 mg *Customer requested unpurified peptide**

Storage : **Keep Refrigerated**

Remarks: Not for Human Use. Research Purposes Only.

Release By: _____

Jaswinder Kaur

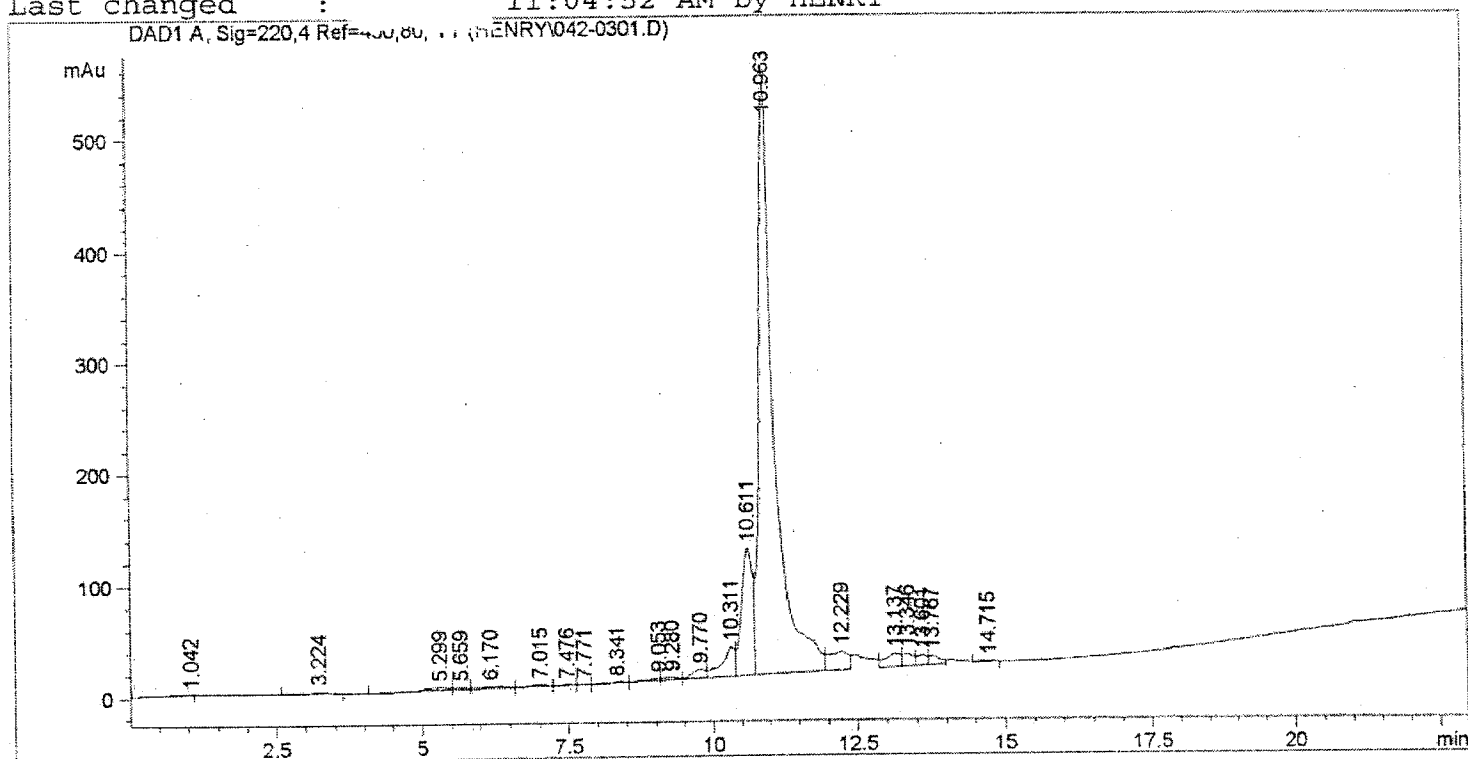
Date: _____

Quality Control

Data File C:\HPCHEM\1\DATA\HENRY\042-0301.D

Sample Name: AA66.1

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=====
Injection Date   :       7:47:03 PM          Seq. Line :    3
Sample Name      : AA66.1                   Vial       :   42
Acq. Operator    : HENRY                    Inj         :    1
                                           Inj Volume  : 5 µl
Different Inj Volume from Sequence !      Actual Inj Volume : 2 µl
Sequence File    : C:\HPCHEM\1\SEQUENCE\DEF_LC.S
Method           : C:\HPCHEM\1\METHODS\0-100-20.M
Last changed     :       11:04:52 AM by HENRY
=====
```



```
=====
                          Area Percent Report
=====
```

```
Sorted By           :      Signal
Multiplier          :      1.0000
Dilution            :      1.0000
```

Signal 1: DAD1 A, Sig=220,4 Ref=450,80, TT
Results obtained with standard integrator!

Peak #	RetTime [min]	Type	Width [min]	Area [mAu*s]	Height [mAu]	Area %
1	1.042	BV	3.5187	29.64368	1.00569e-1	0.2185
2	3.224	BV	0.2008	29.17170	1.88686	0.2151
3	5.299	BV	0.3742	87.51175	2.88747	0.6452
4	5.659	VV	0.2618	37.78679	2.13710	0.2786
5	6.170	VV	0.4283	68.21159	1.91198	0.5029
6	7.015	VV	0.2753	40.34123	1.83774	0.2974
7	7.476	VV	0.1824	12.75530	9.23668e-1	0.0940
8	7.771	VV	0.1100	3.22723	3.85199e-1	0.0238
9	8.341	PV	0.1796	12.61311	1.15197	0.0930
10	9.053	PV	0.2286	44.93953	2.49896	0.3313
11	9.280	VV	0.2323	45.01051	2.51241	0.3318

from Page No. Start

PRISM Matrix ELISA G Assay

Date/Initials

W. Scott

Reagents and Supplies

- Nunc Polysorp 96 well immuno-plate, Nunc cat#62409-005 batch# 088642
 - PBS pH 7.4 (phosphate buffered saline, 8g NaCl, 0.29g KCl, 1.44g Na_2HPO_4 , 0.24g KH_2PO_4 , add H_2O to 1L and pH 7.4; 0.2 μ filter) AVC lot# 97-88-2
 - Assay Buffer: 2% BSA in PBS (20g of bovine serum albumin per liter PBS, fraction V, ICN Biomedicals, cat#IC15142983 AVC lot# 97-111-3)
 - Goat anti-GST polyclonal Ab, stock 5 mg/ml, stored at 4°C, Amersham Pharmacia cat#27-4577-01, lot# 191545
 - Dilute 1:1000 in PBS, final concentration 5 μ g/ml. Date prepared 10-11-01
 - HRP-Streptavidin, 2.5mg/2ml stock stored @ 4°C, Zymed cat#43-4323, lot# 16113-01
 - dilute 1:2000 into Assay buffer, final [0.5 μ g/ml]
 - Wash Buffer, 0.2% Tween 20 in 50mM Tris pH 8.0, AVC lot# 97-102-02
 - Biotinylated peptides (HPLC purified, stock solution store in -20°C freezer #7)
 - GST-PRISM proteins (stock stored @ -80°C, after 1st thaw store in -10°C freezer #7)
 - TMB (3,3',5,5'-tetramethylbenzidine), ready to use, Dako cat#S1600, lot# 09160
 - 0.18M H_2SO_4 , Sigma cat#S1526, AVC lot# 97-85-01
 - 12-w multichannel pipettor & tips
 - 50 ml reagent reservoirs, Costar#4870
 - 50, 15 ml polypropylene conical tubes
 - Costar Transtar 96 Costar#7605
 - Transtar 96 Cartridge Costar#7610
 - Transtar Costar#
 - Cluster tubes
 - Molecular Devices microplate reader (450 & 650 nm filters)
 - SoftMax Pro software
- *When using reagents stored at or 4°C or -20°C, remove & keep on ice

Protocol

- Coat plate with 100 μ l of 5 μ g/ml anti-GST, O/N @ 4°C
- Dump contents of plate & out tap dry on paper towels
- Add 200 μ l Assay Buffer for 2 hrs at 4°C
- Prepare proteins and peptides in Assay Buffer
- Wash 3X with cold PBS*
- Add proteins at 50 μ l per well, incubate 1 to 2 hrs at 4°C
- Wash 3X with cold PBS*
- Add peptides at 50 μ l per well on ice (write time on plate)
- Incubate on ice after last peptide has been added for exactly 10 minutes
- Place at room temp for exactly 20 minutes
- Prepare HRP-Streptavidin within 10 minutes of time of use
- Promptly wash 3X with cold PBS
- Add 100 μ l per well of HRP-Streptavidin (write time on plate)
- Incubate at 4°C for exactly 20 minutes
- Turn on plate reader and prepare files (store as 0105011.kt1)
- Promptly wash 5X with Wash Buffer
- Add 100 μ l/well TMB substrate (write time on plate)
- Incubate in dark at room temp for a maximum of 30 minutes
- Check plate periodically; if necessary take early readings at 650 nm
- stop reaction with 100 μ l of 0.18M H_2SO_4 , 30 min. after adding TMB
- Take last reading at 450 nm soon after stopping reaction
 - Leave last PBS in wells until ready for next step, i.e. do not let plates dry out

PEPTIDE

	1	2	3	4	5	6	1	2	3	4	5	6
	1	2	3	4	5	6	7	8	9	10	11	12
A	PROTEIN 1											
B	PROTEIN 2											
C	PROTEIN 3											
D	PROTEIN 4											
E	PROTEIN 5											
F	PROTEIN 6											
G	GST + LINKER CONTROL											
H	STANDARD CURVE						STANDARD CURVE					

Glucose	1, 7	2, 8	3, 9	4, 10	5, 11	6, 12
KIAA1634(1)(35-2)	←		0.1 μ g/ml			→
DNAM-1 (AA22L)	100 μ M	10 μ M	1 μ M	0.1 μ M	0.01 μ M	0.001 μ M

To Page No. 25

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

Amber Mosters

Project No. _____

Book No. 164 TITLE 175x6 13AFrom Page No. 27ELISA Plate
Arbor Vita Corp. CONFIDENTIAL

No. 1721

Creation Date 17:33:11 Creation Time 164 Page Number 24 Template 07 Wavelength 450

Col	Row	Protein	Domain(s)	Protein	Protein	Peptide	Lot #	Peptide	Active Substances	AS Comp.	OD
1	A	780AA1854	2	1	1	AAT7.VMPV ES 67 (yeast)	887	10	0.177		
2	A	780AA1854	2	1	1	AAT7.VMPV ES 33 (yeast)	876	10	0.279		
3	A	780AA1854	2	1	1	AAT7.VMPV ES 58 (yeast)	877	10	0.281		
4	A	780AA1854	2	1	1	AAT7.VMPV ES 33 (yeast)	760	10	0.098		
5	A	780AA1854	2	1	1	AAT7.VMPV ES 67 (yeast)	882	10	0.130		
6	A	780AA1854	2	1	1	AAT7.VMPV ES 33 (yeast)	888	10	0.297		
7	A	780AA1854	2	1	1	AAT7.VMPV ES 67 (yeast)	887	10	0.172		
8	A	780AA1854	2	1	1	AAT7.VMPV ES 33 (yeast)	876	10	0.232		
9	A	780AA1854	2	1	1	AAT7.VMPV ES 58 (yeast)	877	10	0.128		
10	A	780AA1854	2	1	1	AAT7.VMPV ES 33 (yeast)	760	10	0.132		
11	A	780AA1854	2	1	1	AAT7.VMPV ES 67 (yeast)	882	10	0.211		
12	A	780AA1854	2	1	1	AAT7.VMPV ES 33 (yeast)	888	10	0.348		
13	A	780AA1854	2	2	2	AAT7.VMPV ES 67 (yeast)	887	10	0.112		
14	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	876	10	0.144		
15	A	780AA1854	2	2	2	AAT7.VMPV ES 58 (yeast)	877	10	0.068		
16	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	760	10	0.098		
17	A	780AA1854	2	2	2	AAT7.VMPV ES 67 (yeast)	882	10	0.109		
18	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	888	10	0.107		
19	A	780AA1854	2	2	2	AAT7.VMPV ES 58 (yeast)	877	10	0.101		
20	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	760	10	0.135		
21	A	780AA1854	2	2	2	AAT7.VMPV ES 67 (yeast)	887	10	0.266		
22	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	876	10	0.089		
23	A	780AA1854	2	2	2	AAT7.VMPV ES 58 (yeast)	877	10	0.213		
24	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	760	10	0.089		
25	A	780AA1854	2	2	2	AAT7.VMPV ES 67 (yeast)	882	10	0.139		
26	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	888	10	0.279		
27	A	780AA1854	2	2	2	AAT7.VMPV ES 58 (yeast)	877	10	0.041		
28	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	760	10	0.093		
29	A	780AA1854	2	2	2	AAT7.VMPV ES 67 (yeast)	882	10	0.138		
30	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	888	10	0.109		
31	A	780AA1854	2	2	2	AAT7.VMPV ES 58 (yeast)	877	10	0.117		
32	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	760	10	0.165		
33	A	780AA1854	2	2	2	AAT7.VMPV ES 67 (yeast)	882	10	0.295		
34	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	888	10	0.022		
35	A	780AA1854	2	2	2	AAT7.VMPV ES 58 (yeast)	877	10	0.137		
36	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	760	10	0.238		
37	A	780AA1854	2	2	2	AAT7.VMPV ES 67 (yeast)	882	10	0.117		
38	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	888	10	0.189		
39	A	780AA1854	2	2	2	AAT7.VMPV ES 58 (yeast)	877	10	0.297		
40	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	760	10	0.485		
41	A	780AA1854	2	2	2	AAT7.VMPV ES 67 (yeast)	882	10	0.101		
42	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	888	10	0.203		
43	A	780AA1854	2	2	2	AAT7.VMPV ES 58 (yeast)	877	10	0.102		
44	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	760	10	0.167		
45	A	780AA1854	2	2	2	AAT7.VMPV ES 67 (yeast)	882	10	0.246		
46	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	888	10	0.484		
47	A	780AA1854	2	2	2	AAT7.VMPV ES 58 (yeast)	877	10	0.128		
48	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	760	10	0.128		
49	A	780AA1854	2	2	2	AAT7.VMPV ES 67 (yeast)	882	10	0.128		
50	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	888	10	0.089		
51	A	780AA1854	2	2	2	AAT7.VMPV ES 58 (yeast)	877	10	0.146		
52	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	760	10	0.32		
53	A	780AA1854	2	2	2	AAT7.VMPV ES 67 (yeast)	882	10	0.207		
54	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	888	10	0.136		
55	A	780AA1854	2	2	2	AAT7.VMPV ES 58 (yeast)	877	10	0.239		
56	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	760	10	0.261		
57	A	780AA1854	2	2	2	AAT7.VMPV ES 67 (yeast)	882	10	0.096		
58	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	888	10	0.194		
59	A	780AA1854	2	2	2	AAT7.VMPV ES 58 (yeast)	877	10	0.096		
60	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	760	10	0.096		
61	A	780AA1854	2	2	2	AAT7.VMPV ES 67 (yeast)	882	10	0.174		
62	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	888	10	0.014		
63	A	780AA1854	2	2	2	AAT7.VMPV ES 58 (yeast)	877	10	0.014		
64	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	760	10	0.014		
65	A	780AA1854	2	2	2	AAT7.VMPV ES 67 (yeast)	882	10	0.014		
66	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	888	10	0.014		
67	A	780AA1854	2	2	2	AAT7.VMPV ES 58 (yeast)	877	10	0.014		
68	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	760	10	0.014		
69	A	780AA1854	2	2	2	AAT7.VMPV ES 67 (yeast)	882	10	0.014		
70	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	888	10	0.014		
71	A	780AA1854	2	2	2	AAT7.VMPV ES 58 (yeast)	877	10	0.014		
72	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	760	10	0.014		
73	A	780AA1854	2	2	2	AAT7.VMPV ES 67 (yeast)	882	10	0.014		
74	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	888	10	0.014		
75	A	780AA1854	2	2	2	AAT7.VMPV ES 58 (yeast)	877	10	0.014		
76	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	760	10	0.014		
77	A	780AA1854	2	2	2	AAT7.VMPV ES 67 (yeast)	882	10	0.014		
78	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	888	10	0.014		
79	A	780AA1854	2	2	2	AAT7.VMPV ES 58 (yeast)	877	10	0.014		
80	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	760	10	0.014		
81	A	780AA1854	2	2	2	AAT7.VMPV ES 67 (yeast)	882	10	0.014		
82	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	888	10	0.014		
83	A	780AA1854	2	2	2	AAT7.VMPV ES 58 (yeast)	877	10	0.014		
84	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	760	10	0.014		
85	A	780AA1854	2	2	2	AAT7.VMPV ES 67 (yeast)	882	10	0.014		
86	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	888	10	0.014		
87	A	780AA1854	2	2	2	AAT7.VMPV ES 58 (yeast)	877	10	0.014		
88	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	760	10	0.014		
89	A	780AA1854	2	2	2	AAT7.VMPV ES 67 (yeast)	882	10	0.014		
90	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	888	10	0.014		
91	A	780AA1854	2	2	2	AAT7.VMPV ES 58 (yeast)	877	10	0.014		
92	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	760	10	0.014		
93	A	780AA1854	2	2	2	AAT7.VMPV ES 67 (yeast)	882	10	0.014		
94	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	888	10	0.014		
95	A	780AA1854	2	2	2	AAT7.VMPV ES 58 (yeast)	877	10	0.014		
96	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	760	10	0.014		
97	A	780AA1854	2	2	2	AAT7.VMPV ES 67 (yeast)	882	10	0.014		
98	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	888	10	0.014		
99	A	780AA1854	2	2	2	AAT7.VMPV ES 58 (yeast)	877	10	0.014		
100	A	780AA1854	2	2	2	AAT7.VMPV ES 33 (yeast)	760	10	0.014		

ELISA Plate
Arbor Vita Corp. CONFIDENTIAL

Plate # 1722

Creation Date 17:33:11 Creation Time 164 Page Number 24 Template 08 Wavelength 450

Col	Row	Protein	Domain(s)	Protein	Protein	Peptide	Lot #	Peptide	Active Substances	AS Comp.	OD
1	A	80PCE-75	2	1	1	AAT7.VMPV ES 67 (yeast)	887	10	0.114		
2	A	80PCE-75	2	1	1	AAT7.VMPV ES 33 (yeast)	876	10	0.183		
3	A	80PCE-75	2	1	1	AAT7.VMPV ES 58 (yeast)	877	10	0.288		
4	A	80PCE-75	2	1	1	AAT7.VMPV ES 33 (yeast)	760	10	0.071		
5	A	80PCE-75	2	1	1	AAT7.VMPV ES 67 (yeast)	882	10	0.087		
6	A	80PCE-75	2	1	1	AAT7.VMPV ES 33 (yeast)	888	10	0.079		
7	A	80PCE-75	2	1	1	AAT7.VMPV ES 58 (yeast)	877	10	0.191		
8	A	80PCE-75	2	1	1	AAT7.VMPV ES 33 (yeast)	760	10	0.076		
9	A	80PCE-75	2	1	1	AAT7.VMPV ES 67 (yeast)	882	10	0.071		
10	A	80PCE-75	2	1	1	AAT7.VMPV ES 33 (yeast)	888	10	0.080		
11	A	80PCE-75	2	1	1	AAT7.VMPV ES 58 (yeast)	877	10	0.068		
12	A	80PCE-75	2	1	1	AAT7.VMPV ES 33 (yeast)	760	10	0.171		
13	A	80PCE-75	2	1	1	AAT7.VMPV ES 67 (yeast)	882	10	0.229		
14	A	80PCE-75	2	1	1	AAT7.VMPV ES 33 (yeast)	877	10	0.423		
15	A	80PCE-75	2	1	1	AAT7.VMPV ES 58 (yeast)	877	10	0.087		
16	A	80PCE-75	2	1	1	AAT7.VMPV ES 33 (yeast)	760	10	0.189		
17	A	80PCE-75	2	1	1	AAT7.VMPV ES 67 (yeast)	882	10	0.106		
18	A	80PCE-75	2	1	1	AAT7.VMPV ES 33 (yeast)	888	10	0.182		
19	A	80PCE-75	2	1	1	AAT7.VMPV ES 58 (yeast)	877	10	0.354		
20	A	80PCE-75	2	1	1	AAT7.VMPV ES 33 (yeast)	760	10	0.447		
21	A	80PCE-75	2	1	1	AAT7.VMPV ES 67 (yeast)	882	10	0.409		
22	A										

From Page No. X

PRISM Matrix ELISA G Assay

Date/Initials 8/7/01 KC, BK

Reagents and Supplies

- Nunc Polysorp 96 well Immuno-plate, Nunc cat#62409-005 batch# 045987
 - PBS pH 7.4 (phosphate buffered saline, 8g NaCl, 0.29g KCl, 1.44g Na₂HPO₄, 0.24g KH₂PO₄, add H₂O to 1L and pH 7.4; 0.2 µ filter) AVC lot# 97-93-04
 - Assay Buffer: 2% BSA in PBS (20g of bovine serum albumin per liter PBS, fraction V, ICN Biomedicals, cat#IC15142983 AVC lot# 97-100-02
 - Goat anti-GST polyclonal Ab, stock 5 mg/ml, stored at 4°C, Amersham Pharmacia cat#27-4577-01, lot# 191546
 - Dilute 1:1000 in PBS, final concentration 5 µg/ml. Date prepared 8/7/01
 - HRP-Streptavidin, 2.5mg/2ml stock stored @ 4°C, Zymed cat#43-4323, lot# 10162403 dilute 1:2000 into Assay buffer, final [0.5 µg/ml]
 - Wash Buffer, 0.2% Tween 20 in 50mM Tris pH 8.0, AVC lot# 97-96-02
 - Biotinylated peptides (HPLC purified, stock solution store in -20°C freezer #7)
 - GST-PRISM proteins (stock stored @ -80°C, after 1st thaw store in -10°C freezer #7)
 - TMB (3,3',5,5', tetramethylbenzidine), ready to use, Dako cat#S1600, lot# 07160
 - 0.18M H₂SO₄, Sigma cat#S1526, AVC lot# 97-96-03
 - 12-w multichannel pipettor & tips
 - 50 ml reagent reservoirs, Costar#4870
 - 50, 15 ml polypropylene conical tubes
 - Costar Transstar 96 Costar#7605
 - Transstar 96 Cartridge Costar#7610
 - Transstar Costar#
 - Cluster tubes
 - Molecular Devices microplate reader (450 & 650 nm filters)
 - SoftMax Pro software
- *When using reagents stored at or 4°C or -20°C, remove & keep on ice

Protocol

- Coat plate with 100 µl of 5 µg/ml anti-GST, O/N @ 4°C
- Dump contents of plate & out tap dry on paper towels
- Add 200 µl Assay Buffer for 2 hrs at 4°C
- Prepare proteins and peptides in Assay Buffer
- Wash 3X with cold PBS*
- Add proteins at 50 µl per well, incubate 1 to 2 hrs at 4°C
- Wash 3X with cold PBS*
- Add peptides at 50 µl per well on ice (write time on plate)
- Incubate on ice after last peptide has been added for exactly 10 minutes
- Place at room temp for exactly 20 minutes
- Prepare HRP-Streptavidin within 10 minutes of time of use
- Promptly wash 3X with cold PBS
- Add 100 µl per well of HRP-Streptavidin (write time on plate)
- Incubate at 4°C for exactly 20 minutes
- Turn on plate reader and prepare files (store as 0105011k1)
- Promptly wash 5X with Wash Buffer
- Add 100 µl/well TMB substrate (write time on plate)
- Incubate in dark at room temp for a maximum of 30 minutes
- Check plate periodically; if necessary take early readings at 650 nm
- stop reaction with 100 µl of 0.18M H₂SO₄, 30 min. after adding TMB
- Take last reading at 450 nm soon after stopping reaction
 - Leave last PBS in wells until ready for next step, i.e. do not let plates dry out

PEPTIDE

	1	2	3	4	5	6	1	2	3	4	5	6
	1	2	3	4	5	6	7	8	9	10	11	12
A	PROTEIN 1											
B	PROTEIN 2											
C	PROTEIN 3											
D	PROTEIN 4											
E	PROTEIN 5											
F	PROTEIN 6											
G	GST + LINKER CONTROL											
H	STANDARD CURVE						STANDARD CURVE					

Standard Curve						
Column	1, 7	2, 8	3, 9	4, 10	5, 11	6, 12
PSD95(1) #143.1	5 µg/ml					
Tax AA56L	5 µM	1.19 µM	0.283 µM	0.067 µM	0.016 µM	0.004 µM

186 x 6

183 x 6

36.2 } → Neurotrophin - differently made
36.3 } biotinylated peptides for comparison
80.1 HPV 26
215 HIV
200 Serotonin
258 Noradrenaline

Witnessed & Understood by me,

Marjorie Jones

Date

Invented by

Recorded by

C. Jones

Date

To Page No. 8

Book No. 157

TITLE
$$186 \times 611 \text{ N}$$

From Page No 86

Arbor Vita Corp. CONFIDENTIAL

ELISA Plate
Arbor Vita Corp. CONFIDENTIAL

Plate 2 1385

Creation Date	Creation Time	Notebook	Page Number	Template	Wavelength
22-02-20	18:27	23	02	450	

Col	Row	Protein	Domain	Seq	Prot. Name	Peptide	Seq	Peptide	Active Substance	AS Comp.	DB
1	1	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
2	2	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
3	3	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
4	4	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
5	5	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
6	6	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
7	7	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
8	8	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
9	9	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
10	10	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
11	11	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
12	12	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
13	13	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
14	14	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
15	15	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
16	16	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
17	17	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
18	18	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
19	19	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
20	20	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
21	21	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
22	22	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
23	23	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
24	24	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
25	25	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
26	26	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
27	27	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
28	28	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
29	29	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
30	30	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
31	31	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
32	32	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
33	33	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002
34	34	7760A1834	3	2	AASL3Shenounglin (R181)	854	10	0.002	0.002	0.002	0.002

To Page No. 88

tnessed & Understood by me,

Date

Invented by

Date _____

Recorded by

Page No. X

PRISM Matrix ELISA G Assay

Initials KC, BK

Reagents and Supplies

Nunc Polysorp 96 well Immuno-plate, Nunc cat#62409-005 batch# 045987
PBS pH 7.4 (phosphate buffered saline, 8g NaCl, 0.29g KCl, 1.44g Na₂HPO₄, 0.24g KH₂PO₄, add H₂O to 1L and pH 7.4; 0.2 µ filter) AVC lot# 97-93-04
Assay Buffer: 2% BSA in PBS (20g of bovine serum albumin per liter PBS, fraction V, CN Biomedicals, cat#C15142983 AVC lot# 97-94-01 -04
Boat anti-GST polyclonal Ab, stock 5 mg/ml, stored at 4°C, Amersham Pharmacia cat#27-4577-01, lot# 196545
• Dilute 1:1000 in PBS, final concentration 5 µg/ml. Date prepared _____
HRP-Streptavidin, 2.5mg/2ml stock stored @ 4°C, Zymed cat#43-4323, lot# 10162409
Dilute 1:2000 into Assay buffer, final [0.5 µg/ml]
Wash Buffer, 0.2% Tween 20 in 50mM Tris pH 8.0, AVC lot# 97-87-3
Biotinylated peptides (HPLC purified, stock solution store in -20°C freezer #7)
GST-PRISM proteins (stock stored @ -80°C, after 1" thaw store in -10°C freezer #7)
TMB (3,3',5,5', tetramethylbenzidine), ready to use, Dako cat#S1600, lot# 07160
0.18M H₂SO₄, Sigma cat.#S1526, AVC lot# 97-86-01
12-w multichannel pipettor & tips
50 ml reagent reservoirs, Costar#4870
50, 15 ml polypropylene conical tubes
Costar Transtar 96 Costar#7605
Transtar 96 Cartridge Costar#7610
Transtar Costar#
Cluster tubes
Molecular Devices microplate reader (450 & 650 nm filters)
SoftMax Pro software
Icen using reagents stored at or 4°C or -20°C, remove & keep on ice

Protocol

Cost plate with 100 µl of 5 µg/ml anti-GST, O/N @ 4°C
Dump contents of plate & out tap dry on paper towels
Add 200 µl Assay Buffer for 2 hrs at 4°C
Prepare proteins and peptides in Assay Buffer
Wash 3X with cold PBS*
Add proteins at 50 µl per well, incubate 1 to 2 hrs at 4°C
Wash 3X with cold PBS*
Add peptides at 50 µl per well on ice (write time on plate)
Incubate on ice after last peptide has been added for exactly 10 minutes
Place at room temp for exactly 20 minutes
Prepare HRP-Streptavidin within 10 minutes of time of use
Promptly wash 3X with cold PBS
Add 100 µl per well of HRP-Streptavidin (write time on plate)
Incubate at 4°C for exactly 20 minutes
Turn on plate reader and prepare files (store as 0105011kt1)
Promptly wash 5X with Wash Buffer
Add 100 µl/well TMB substrate (write time on plate)
Incubate in dark at room temp for a maximum of 30 minutes
Check plate periodically; if necessary take early readings at 650 nm
stop reaction with 100 µl of 0.18M H₂SO₄, 30 min. after adding TMB
Take last reading at 450 nm soon after stopping reaction
• Leave last PBS in wells until ready for next step,
i.e. do not let plates dry out

PEPTIDE

	1	2	3	4	5	6	1	2	3	4	5	6
	1	2	3	4	5	6	7	8	9	10	11	12
A	PROTEIN 1											
B	PROTEIN 2											
C	PROTEIN 3											
D	PROTEIN 4											
E	PROTEIN 5											
F	PROTEIN 6											
G	GST + LINKER CONTROL											
H	STANDARD CURVE						STANDARD CURVE					

Standard Curve

Column	1, 7	2, 8	3, 9	4, 10	5, 11	6, 12
PSD95(1) #143.1	5 µg/ml					
Tax AA58L	5 µM	1.19 µM	0.283 µM	0.067 µM	0.016 µM	0.004 µM

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Witnessed & Understood by me,

Date

Invented by

Date

Margorie Jones

Recorded by

Lee M. Jones

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[illegible]

To Page No. 82

Accepted & Understood by me,

Date

Invented by

Date

Recorded by

Recorded by
DeMunne Belmont